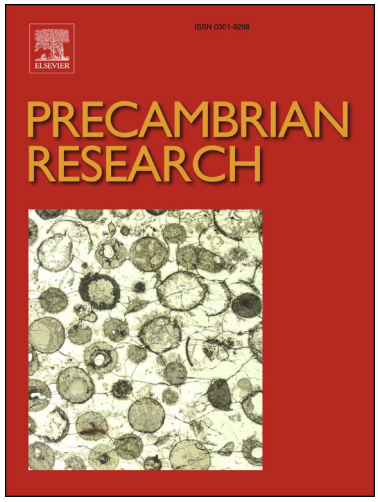

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Environmental control on microbial diversification and methane production in the Mesoarchean

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Abstract

Multiple lines of evidence have revealed a thriving marine biosphere capable of diverse metabolic strategies back to at least 3.5 billion years ago (Ga). However, little is known about microbial ecosystems in lakes and rivers during the Mesoarchean and their role in the evolution of the biosphere. Here we report new carbon and nitrogen isotopic data from the fluvio-lacustrine Lalla Rookh Sandstone in Western Australia (~3.0 Ga) – one of the oldest known non-marine sedimentary deposits. Organic $\delta^{13}C$ values (-30‰ to -38‰) are best interpreted as recording carbon fixation by methanogens using the acetyl CoA pathway mixed with organisms using the Calvin cycle, while $\delta^{15}N$ data (0‰ to -1‰) likely reflect biological N_2 fixation using FeMo-nitrogenase. When compared with data from the literature, we show that lacustrine habitats of Mesoarchean age (3.2-2.8 Ga) are systematically depleted in $\delta^{13}C$ (-37 ± 5‰) relative to marginal marine (-32 ± 7‰) and open marine settings (-27 ± 3‰), suggesting that methanogenesis was relatively more important in lacustrine communities. Our findings highlight: (a) the widespread use of biological N_2 fixation by the Mesoarchean biosphere, (b) the potential importance of continental habitats for methane production and perhaps for the formation of hydrocarbon haze, and (c) the possible role of land masses in driving microbial diversification on the early Earth.

1. Introduction

Life in Earth's oceans dates back to at least 3.5 Ga and possibly 3.8 Ga (Buick, 2007; Nisbet and Fowler, 2014). The strongest evidence is based on occurrences of stromatolites (Buick et al., 1981; Walter et al., 1980) and isotopic fractionations of carbon and sulfur that collectively point to a diverse early ecosystem capable of phototrophy, CO_2 fixation, methanogenesis, and sulfate reduction (Schidlowski, 2001; Shen et al., 2001; Ueno et al., 2006). In contrast, non-marine ecosystems of Mesoarchean age are much less well characterized. Recent reports demonstrated the presence of microbial sulfate reducers in a Mesoarchean paleosol of the Moodies Group at ~3.2 Ga (Nabhan et al., 2016), and carbon isotope data from kerogenous fluvial sediments of the Witwatersrand Supergroup suggest microbial methane cycling at 2.8-2.9 Ga (Guy et al., 2012). However, it is unknown if Mesoarchean continents were as widely inhabited as they are today, if terrestrial ecosystems were systematically distinct from their marine counterparts, and if these settings could have influenced global trends in biological evolution or climate. Given the resilience of modern microorganisms under harsh conditions (e.g. Sinha and Häder, 2008; Warren-Rhodes et al., 2006), it is likely that life was able to inhabit Archean land masses (Nisbet and Fowler, 2014), but the metabolic properties of this biosphere are not well known.

Differences between marine and non-marine ecosystems have been inferred from the Neoarchean rock record (2.8-2.5 Ga), where shallow marine and non-marine facies show markedly more negative organic carbon isotope values than open-ocean sediments, which has been interpreted as evidence of higher oxygen availability and methanotrophy in continental settings (Eigenbrode and Freeman, 2006; Flannery et al., 2016). For the Neoarchean, this interpretation is consistent with increasing evidence for temporary and/or localized appearances of free O_2 in surface environments at this time (Anbar et al., 2007; Kendall et al., 2010; Siebert et al., 2005; Stüeken et al., 2017; Wille et al., 2007). In the Mesoarchean, however, evidence of biological O_2 production is scarce (Crowe et al., 2013; Homann et al., 2015; Planavsky et al., 2014; Riding et al., 2014; Stüeken et al., 2012). In a more homogeneous redox landscape, microbial ecology may have been distinct from later time periods. To test this hypothesis of possibly fewer metabolic interactions, we analyzed samples from the fluvio-lacustrine Lalla Rookh Sandstone in Western Australia (~3.0 Ga, Fig. 1). This unit represents one of the oldest

known lacustrine sedimentary deposits. We performed organic carbon, total nitrogen, carbonate carbon and oxygen isotope analyses to characterize the basic metabolic functions of this terrestrial ecosystem. When combined with literature data from other Mesoarchean marine and non-marine sediments, we conclude that non-marine and marginal marine habitats in the Mesoarchean hosted distinctive microbial communities with a higher proportion of methanogens compared to oceanic settings. This may have important implications for the fluxes of microbial methane to the atmosphere, as well as for the role of continental growth in microbial diversification.

2. Geologic setting

The Lalla Rookh Sandstone was deposited in a small strike-slip fault-bounded intra-continental basin on the Pilbara craton of Western Australia (Krapež, 1984; Krapež and Barley, 1987) (Fig. 1). It unconformably overlies the volcano-sedimentary Warrawoona Group, the Soanesville Group and granitoid batholiths, all of which have contributed detrital material to the basin. This diverse substrate chemically resembled continental crust and was at least micro-continental in scale. Indeed, if the Vaalbara hypothesis of Kaapvaal-Pilbara cratonic aggregation (Button, 1979; Cheney, 1996) can be extended back to the Paleoarchean (Bradley et al., 2015), then the basement to the Lalla Rookh Sandstone was already truly continental, providing a comparatively cool, buoyant and stable terrestrial platform. The Lalla Rookh Sandstone itself has only been metamorphosed to sub-greenschist facies, as the immediately overlying terrestrial basalts and sediments of the ~2.77 Ga lower Fortescue Group are of prehnite-pumpellyite grade (Smith et al., 1982) and the Lalla Rookh mudrocks themselves are composed of illite and chlorite (Buick et al., 1998). The age of the Lalla Rookh Sandstone is inferred to be ~3.0 Ga based on tectono-stratigraphic correlation with the 2.99 Ga Whim Creek Group (Barley et al., 1994; Krapež and Barley, 1987).

Sedimentary facies in the Lalla Rookh Sandstone include braided streams, alluvial fans, fan deltas and flood plains with lacustrine deposits (Eriksson, 1981; Eriksson et al., 1994; Krapež, 1984) (Fig. 2). Sediment derived from alluvial fans along the faulted basin margins was transported northeast by braided rivers that flowed into a shallow lake. Of the 3–4.5 km of sedimentary rocks in the basin, most (85%) are trough-cross-bedded sandstones with minor

conglomerate, diamictite and mudrocks (Fig. 3a). Detrital sand grains are predominantly composed of quartz with some microcline, whereas conglomerates contain lithic fragments of chert, iron formation, metasandstone, quartz-mica schist and granitoid representing the underlying basement rocks. Detrital pyrite is present in low abundances in heavy-mineral placers (Fig. 3b) along with siderite (Rasmussen and Buick, 1999), chromite, zircon, monazite and leucoxene after ilmenite (Krapež and Furnell, 1987). The roundness of the pyrite grains, their association with sand and silt-sized siliciclastic grains, and their occurrence in lag deposits with other heavy minerals (Fig. 3c, d) attest to their detrital origin.

The shales from the lacustrine facies, which were the focus of this study, are interfingered and interlaminated with siltstone and very fine-grained sandstone representing shoreface, mud-flat, delta-front, basinal laminite and turbidite sub-facies (Eriksson et al., 1994; Krapež, 1984). The silt and sand grains are partly carbonate-cemented (1–20%, average 6%). Any pyrite present in the mudrocks is overwhelmingly detrital in origin (Fig. 3); we did not observe any syngenetic or diagenetic pyrite in our samples with reflected light microscopy. Planar to undulose kerogen-rich laminae from 0.1–1 mm thick (Fig. 3a) are abundant. A fluvio-lacustrine interpretation of the mudrocks is supported by rapid lateral and vertical facies changes, the presence of fluvial current ripples and lineations interfering with wind wave ripples in the associated sandstones, and the presence of interbedded conglomeratic channel deposits (Krapež, 1984). If this basin had a connection to the ocean, it would have been intermittent and restricted, which would not alter our overall interpretation.

3. Materials and methods

3.1. Sampling

Thirteen samples of the shale-dominated lacustrine facies in the Lalla Rookh Sandstone were obtained from a drill core (SRD-8, Fig. 2b) that was drilled in the northeastern part of the Lalla Rookh basin (Fig. 2a). The company that undertook the drilling campaign (Anaconda Copper) is no longer operational and a core log is not available. These samples are to our knowledge the only accessible unweathered material of the lacustrine facies that contain significant amounts of kerogen. Although the sample set is admittedly small, it offers important

insights into a rarely preserved Mesoarchean habitat that has received very little attention in the geobiological literature.

3.2. Analytical methods

Sample preparation and analyses followed established protocols in the University of Washington Isolab (Stüeken, 2013). The outer surfaces of all rock samples were removed with a rock saw, the rocks were fragmented into sub-cm chips which were washed in a sonic bath with methanol (reagent grade), 2N HCl (reagent grade) and 18 MΩ DI-H_2O. The dry chips were pulverized in an Al_2O_3 ceramic puck mill and stored in pre-combusted scintillation vials. Between samples, the mill was cleaned with silica sand that had been baked overnight at 1100°C.

For nitrogen and organic carbon measurements, a 0.5g aliquot of powder was decarbonated with 6N HCl at 60°C in borosilicate centrifuge tubes. The acid was refreshed twice and washed out with three treatments of 18 MΩ DI-H_2O. De-carbonated powders were weighed into tin capsules and analyzed by flash combustion in an elemental analyzer (Costech) coupled to an isotope-ratio mass spectrometer (Thermo MAT253). For carbonate C and O isotopes, untreated powder aliquots were weighed into glass vials and reacted with concentrated phosphoric acid at 80°C for 10 minutes in a Kiel Carbonate Device coupled to a Thermo Delta Plus IRMS. All isotopic data were corrected by standard three-point calibration (Coplen et al., 2006). Average reproducibility, expressed as 1 SD (standard deviation), was 0.3‰ for $\delta^{15}N$, 0.2‰ for $\delta^{13}C_{org}$, 0.03‰ for $\delta^{13}C_{carb}$ and 0.1‰ for $\delta^{18}O_{carb}$. Long-term reproducibility of N and C_{org}, tested with our in-house rock standard UW-McRae, was 0.3‰ for both. Results are expressed in delta notation relative to atmospheric air for $\delta^{15}N$ and relative to VPDB for $\delta^{13}C$ and $\delta^{18}O$.

4. Results

Organic carbon isotopes in the Lalla Rookh samples fall between -37.6‰ and -30.3‰, while total nitrogen isotopes range from -1.3‰ to -0.1‰ (Table 1, Fig. 4a). Total organic carbon (TOC, avg. 0.3 ± 0.2%) and total nitrogen (avg. 41 ± 9 ppm) are strongly correlated ($r^2 = 0.81$), but the regression shows a positive intercept at 25ppm TN (Fig. 5a). Atomic C/N ratios are 73 ±

25 on average and uncorrelated with $\delta^{15}N_{bulk}$ (Fig. 5b). Carbonate cement has low $\delta^{13}C_{carb}$ values between -3.8‰ and -9.2‰ and very low $\delta^{18}O$ values between -17.8‰ and -20.4‰ (Table 2, Fig. 4b). The two parameters are uncorrelated ($r^2 = 0.35$, Fig. 6).

5. Discussion

5.1. Diagenesis and metamorphism

Carbon, nitrogen and oxygen isotopic ratios can be subject to diagenetic and metamorphic overprinting. In particular $\delta^{18}O_{carb}$ is very easily reset by percolating fluids. Our $\delta^{18}O_{carb}$ data overlap well with the broader Mesoarchean $\delta^{18}O_{carb}$ record for which widespread alteration has been proposed (Jaffrés et al., 2007). The weak correlation between $\delta^{18}O_{carb}$ and $\delta^{13}C_{carb}$ may suggest that the two parameters were not altered by the same process. Our $\delta^{13}C_{carb}$ values plot below average marine carbonate of Mesoarchean age (Krissansen-Totton et al., 2015) (Fig. 4b), consistent with alteration during diagenesis or metamorphism. During metamorphism, carbonate-bound carbon can re-equilibrate with isotopically more negative organic carbon, but this process only becomes significant (>3‰) at greenschist facies (Hayes et al., 1983; Schidlowski, 1987). If so, the most carbonate-poor samples should be the most affected and thus show the most negative $\delta^{13}C_{carb}$ values, which is the opposite of what is observed. Our measurements of $\delta^{13}C_{carb}$ and carbonate content are not correlated ($r^2 = 0.36$) and the two most carbonate-rich samples (14-21%) show among the lowest $\delta^{13}C_{carb}$ values (Table 2). It is thus more likely that the negative $\delta^{13}C_{carb}$ data result not from metamorphism but from diagenetic processes whereby HCO_3^- ions with negative $\delta^{13}C$ were generated by oxidation of organic matter. Diagenetic processes in anoxic sediments can decrease $\delta^{13}C_{org}$ by 1-2‰ (Lehman et al., 2002) or more if biomass is degraded by organisms using the acetyl-CoA pathway (Londry et al., 2008). This could conceivably have occurred in the Lalla Rookh sediments. Nitrogen isotopes have been observed to approach values around 0‰ during anoxic diagenesis, which is attributed to the growth of N_2-fixing microbes within sediments (Lehman et al., 2002). Such small diagenetic imprints on the organic C and N data would still be consistent with our environmental interpretation (Section 5.3).

Regional metamorphic alteration of organic carbon tends to drive organic matter isotopically more positive, but as for carbonate this process is insignificant below greenschist facies (Hayes et al., 1983; Schidlowski, 1987). The same is true for $\delta^{15}N$ values, which can increase during metamorphism as ^{14}N is lost preferentially (Haendel et al., 1986). This process is associated with increasing C/N ratios (Haendel et al., 1986). However, the effect is small (<1‰) below greenschist facies (Thomazo and Papineau, 2013), and we did not find a correlation between $\delta^{15}N$ and C/N ratios in our dataset ($r^2 < 10^{-4}$). It is also unlikely that our data have been compromised by contact metamorphism and associated fluid migration as described from other settings (Schimmelmann and Lis, 2010; Schimmelmann et al., 2009), because this process results in a strong anti-correlation between $\delta^{15}N$ and $\delta^{13}C_{org}$, which is not the case in our dataset (r^2 = 0.17, Fig. 5d). Significant metamorphic resetting of carbon and nitrogen isotopic ratios is therefore unlikely.

5.2. Detrital contributions

Most N in sedimentary rocks is either bound to organic matter or substituted for K in silicate minerals. A large fraction of silicate-bound N likely forms during diagenesis, when NH_4^+ is released from degrading biomass (Boudreau and Canfield, 1988) and incorporated into clay minerals (Palya et al., 2011; Schroeder and McLain, 1998). In this case, the silicate-bound N would still be part of the original authigenic component that records isotopic information about the ecosystem. It is important to note that diagenetic biomass degradation via sulfate reduction or methanogenesis would remove organic C as CO_2 or CH_4 whereas organic N would stay behind as NH_4^+, because these anaerobic metabolisms are not energetic enough to oxidize organic N to N_2 or NO_3^- (Stüeken et al., 2016). Diagenetic alteration could thus lead to an excess of N in sediments relative to organic C, which may explain the positive y-axis intercept in Fig. 5a. However, it is conceivable that some of this N excess results instead from allochthonous N input via detrital silicate minerals. If so, then the measured bulk isotopic composition would represent a mixture between the authigenic and allochthonous phase. To test if a potential detrital N contribution could impact our results, we performed a mass balance calculation to reconstruct the $\delta^{15}N$ value of authigenic N in our samples. We assumed that the detrital phase is equal to the y-axis intercept in Fig. 5a ($N_{detrital}$ = 25 ± 3 ppm), which is a conservative assumption, as noted

above. We further assumed that the detrital phase has an isotopic composition of $\delta^{15}N_{detrital}$ = -1.2 ± 0.4‰, which we derived from the regression in Fig. 5c, after removing one outlier. The authigenic component ($\delta^{15}N_{auth}$) was then calculated as follows (Eq. 1):

$$\delta^{15}N_{authigenic} = (TN \times \delta^{15}N_{bulk} - N_{detrital} \times \delta^{15}N_{detrital}) / (TN - N_{detrital}) \quad \text{(Eq. 1)}$$

The results shown in Fig. 7 fall between -1.4‰ and +1.5‰ and are thus only marginally different from uncorrected $\delta^{15}N_{bulk}$ values (-1.3‰ to -0.1‰). We therefore conclude that a potential contribution of detrital silicate-bound N would not have significantly perturbed the primary isotopic signal.

5.3. Carbon and nitrogen cycling in the Lalla Rookh Sandstone

Our nitrogen isotopic data – with and without correcting for a detrital component (Fig. 7) – fall in the range imparted by microbial nitrogen fixation using the FeMo nitrogenase enzyme (Zhang et al., 2014). These values are inconsistent with large reservoirs of dissolved nitrate or ammonium, which would be subject to processes such as denitrification, anammox or partial assimilation that impart large isotopic fractionations of several permil or more (reviewed by Stüeken et al., 2016). The results thus corroborate previous evidence for the early evolution of biological N_2 fixation and for low abundances of dissolved nitrogenous ions in Mesoarchean aquatic environments (Stüeken et al., 2015). This conclusion is further supported by new phylogenetic evidence for the presence of nitrogenase in the last universal common ancestor (Weiss et al., 2016). The inferred availability of Mo in a non-marine setting may indicate at least some local oxidative weathering, perhaps beneath phototrophic microbial mats that could have been producing local O_2 in an otherwise anoxic atmosphere (Lalonde and Konhauser, 2015; Sumner et al., 2015). As little as a few nM of Mo are sufficient to trigger detectable levels of biological N_2 fixation in modern organisms (Glass et al., 2010; Zerkle et al., 2006).

Oxidative processes may be further indicated by the low $\delta^{13}C_{carb}$ values between -3‰ and -10‰ that are much more negative than the -0.2% ± 1.6‰ average value of marine carbonates from 3.2–2.8 Ga (Krissansen-Totton et al., 2015). Such isotopically negative carbonate cements are most parsimoniously explained by diagenetic remineralization of organic matter as it occurs

in modern rivers and lakes (Mook, 2001). Possible electron acceptors for this reaction could have been ferric iron oxides, which occur in conglomeratic clasts in the Lalla Rookh Sandstone (Krapež, 1984), or low levels of O_2 from oxygenic photosynthesis. Sulfate may have been present, but if so it was at very low concentrations because authigenic pyrite was not observed. The preservation of fine-grained detrital pyrite rules out that sulfide was produced transiently and then underwent re-oxidation by other organisms. Such a re-oxidation process should also have consumed the detrital pyrite fraction.

Relative to atmospheric CO_2 (-7‰, Schidlowski, 2001), the organic carbon isotopes are at or below the minimum limit of -35‰ known for the Rubisco I enzyme in the Calvin-Benson-Bassham (or reductive pentose phosphate) cycle that is used for CO_2 fixation by many phototrophic organisms and some chemotrophs (Nisbet et al., 2007; Schidlowski, 1987; Zerkle et al., 2005). Most biomass of Rubisco-dependent organisms falls around -26 ± 7‰, as also indicated by the marine $\delta^{13}C_{org}$ record through most of Earth's history (Schidlowski, 2001). It is unlikely that the more negative values down to -38‰ were simply the result of Rubisco acting on a more negative CO_2 reservoir compared to atmospheric CO_2, because the Lalla Rookh basin was an open system (as indicated by widespread fluvial deposits) where dissolved CO_2 should have been equilibrated with the large atmospheric CO_2 reservoir. For example, with pCO_2 of 0.1 bar, dissolved CO_2 would have been two orders of magnitude greater than today, making it more difficult for respiratory processes to alter the isotopic composition of this dissolved pool (Slotznick and Fischer, 2016). Isotopically light CO_2 could potentially have been generated within microbial mats; however, if this light CO_2 phase were able make a substantial contribution to the C isotope record, then isotopically light biomass should be pervasive throughout the Precambrian, which is not the case (Krissansen-Totton et al., 2015). The isotopically negative diagenetic carbonate noted above is clearly in disequilibrium with the atmosphere and thus likely unrepresentative of dissolved CO_2 in well-mixed river waters that would have been the primary source for CO_2 fixation. So while Rubisco cannot explain the most negative $\delta^{13}C_{org}$ values, they can easily be generated by the reductive acetyl-CoA (or Wood-Ljungdahl) pathway for CO_2 fixation that is used by some sulfate reducers, autotrophic acetogens and methanogens, including heterotrophic methanogens that degrade biomass of other organisms (Londry et al., 2008). Sulfate concentrations were probably too low in this setting to support microbial sulfate reduction. Even if some sulfate reducers were present, many of these organisms

use other metabolic pathways that impart smaller fractionations in $\delta^{13}C_{org}$ (Londry and Des Marais, 2003). Acetogens may have been present; however, the isotopically very negative acetate that they produce (often fractionated by -55‰ relative to the source bicarbonate, Blaser et al., 2013) should have supplied substrate to heterotrophic microbes whose biomass would have been as negative as the acetate. The absence of very large depletions in $\delta^{13}C_{org}$ may thus argue against significant autotrophic acetogenesis. It is thus most likely that the very low $\delta^{13}C_{org}$ values reflect some form of methanogenesis which can produce biomass more negative than -35‰ regardless of the substrate used (Londry et al., 2008). Varying mixtures between methanogenic, chemotrophic and phototrophic organisms could explain the observed spread in the data. Hence our results, along with the single datum from methane-bearing fluid inclusions at 3.48 Ga (Ueno et al., 2006), support the concept that the acetyl-CoA carbon fixation pathway first evolved well before 3.0 Ga.

5.4. Ecosystem heterogeneity in the Mesoarchean

Combined with mid-Archean carbon isotopic data from the literature (Fig. 8), our results reveal a systematic difference between marine and non-marine/brackish environments, evaluated further with Student t-tests. Marine samples include shales and cherts from continental shelves and deeper basins (Table 3). We classified as brackish those samples that are either deltaic or estuarine (Booysens Fm, 2.87 Ga, and Moodies Gp, 3.22 Ga, Gamper et al., 2012; Guy et al., 2012; Stüeken et al., 2015) or that were deposited in a littoral setting with evidence of freshwater input (Farrel Quartzite, 3.0 Ga, House et al., 2013; Sugahara et al., 2010). Non-marine samples include the lacustrine Lalla Rookh Sandstone (this study) and data from several fluvial units in the Witwatersrand Supergroup (Guy et al., 2012). This classification reveals that $\delta^{13}C_{org}$ values in marine sediments (-27.1 ± 2.9‰) are systematically less negative than those of brackish (-32.3 ± 7.4‰) and non-marine settings (-36.6 ± 5.4‰). The differences between all three data sets are statistically significant ($p_{two\text{-}tailed} < 0.0002$).

It is likely that most if not all metabolic strategies existed at some level in all three types of environments. However, based on these sample sets, the trend suggests that organisms using the acetyl-CoA pathway – and thus capable of producing biomass with $\delta^{13}C$ values below -35‰ – were at least *relatively* more abundant in brackish to non-marine habitats than in the ocean. A

higher proportion of acetyl-CoA-based sulfate reducers in onshore settings is unlikely, because (a) the major source of sulfate to the Mesoarchean ocean was probably volcanic SO_2 photolysis, which would have been spread globally (Huston and Logan, 2004; Stüeken et al., 2012), and (b) as noted above, most sulfate reducers use other carbon-fixation pathways with smaller fractionations in $\delta^{13}C$ (Londry and Des Marais, 2003). Hence the observed environmental trend suggests that the acetyl-CoA pathway was mostly used by methanogens (± acetogens) rather than by sulfate reducers in onshore settings. The relative preference for methanogenesis in continental brackish and freshwater settings may be explained by lesser competition with sulfate reducers for H_2 and organic substrates (e.g. Oremland and Polcin, 1982).

Although the observed gradient from continental to oceanic settings is similar to that observed in the Neoarchean (Eigenbrode and Freeman, 2006; Flannery et al., 2016), lacustrine $\delta^{13}C_{org}$ values in the Mesoarchean do not get anywhere close to the Neoarchean minimum of -60‰. This difference may be due to a much lower proportional abundance of methanotrophic biomass in the Mesoarchean. Methanotrophy requires an electron acceptor such as O_2, Fe^{3+}, NO_3^- or SO_4^{2-}. If so, the observed ecosystem heterogeneity between marine and non-marine Archean settings may have evolved over time in response to the progressive expansion of oxic surface environments.

Lastly, the extension of this ecological gradient back to the Mesoarchean has several important implications. First, a significant methane flux from continental settings may have contributed to the proposed Archean hydrocarbon haze (Arney et al., 2016; Domagal-Goldman et al., 2008; Haqq-Misra et al., 2008). Such a haze forms only at high atmospheric CH_4/CO_2 ratios, and it has been invoked to explain patterns in mass-independent sulfur isotope fractionation (Claire et al., 2014; Domagal-Goldman et al., 2008; Izon et al., 2015; Zerkle et al., 2012). As proposed by Arney et al. (2016), a haze could have facilitated the colonization of land masses, because it would have shielded organisms from harmful UV radiation. A preferential expansion of methanogens into lakes and rivers would thus have acted as a positive feedback for the habitability of non-marine environments. Second, continental habitats evidently offered environmental niches that were distinct from those in the ocean and could thus have fostered genetic diversification. Environmental differences include a greater flux of nutrients, in particular trace metals and phosphorus, that were liberated during weathering (Crowe et al., 2013; Planavsky et al., 2014; Satkoski et al., 2016) and were thus perhaps more bioavailable in

freshwater. The lower salinity may have been preferable for certain life forms as exemplified by the proposition that eukaryotes may have originated in freshwater in the Proteroozic (Sánchez-Baracaldo et al., 2017). Recent models suggest a major increase in the proportion of continental crust around 3 Ga (Dhuime et al., 2015), concurrent with phylogenetic evidence for major biological radiation (David and Alm, 2011). As indicated by our data, the expansion of continental habitats may have played a major role in driving this biological diversification event.

6. Conclusion

Our new data from the fluvio-lacustrine Lalla Rookh Sandstone, along with published contemporaneous datasets, reveal several important aspects of the Mesoarchean biosphere. First, even the oldest known lake deposits in the rock record contain diverse evidence of biological activity, meaning that continents were colonized by microbial consortia back to at least 3 billion years ago. Second, biological N_2 fixation was a widespread mode of nitrogen acquisition in the Mesoarchean biosphere in both non-marine and marine habitats, indicating that fixed nitrogen was low in these settings and redox reactions were suppressed. Thus, the Mesoarchean appears to have represented the "last stand" of largely anaerobic microbiota prior to the Neoarchean emergence of temporary or localized oxygenated conditions that allowed limited expression of aerobic microbial metabolisms. Third, microbial community structures and metabolic strategies were systematically different between marine and brackish to non-marine habitats, despite the apparent absence of a significant redox gradient. Methanogens appear to have contributed a larger proportion of total biomass in lakes and estuarine settings compared to the open ocean. Epicontinental environments may have contributed significantly to atmospheric methane levels and the formation of a hydrocarbon haze with possible implications for climate and continental habitability (Arney et al., 2016; Domagal-Goldman et al., 2008; Haqq-Misra et al., 2008). More importantly, the emergence of land masses around 3 Ga (Dhuime et al., 2015) could have been a major driver of the diversification of early life on Earth (David and Alm, 2011).

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Tables

Table 1: Lalla Rookh organic carbon and total nitrogen isotopic data. Samples were analyzed in duplicate, values quoted are averages. SD = standard deviation, RE = relative error. TOC and TN concentrations are relative to the decarbonated residue.

Sample	TN [ppm]	RE [%]	$\delta^{15}N$ [‰]	SD [‰]	TOC [%]	RE [%]	$\delta^{13}C$ [‰]	SD [‰]	C/N [at]	RE [%]
LallaRookh_1	64	1.8	-1.32	0.27	0.60	0.50	-34.17	0.03	109	1
LallaRookh_2	44	0.3	-0.20	0.10	0.44	1.30	-30.27	0.16	118	2
LallaRookh_3	36	0.3	-0.80	0.56	0.24	1.56	-37.56	0.07	77	1
LallaRookh_5	36	4.9	-0.37	0.03	0.21	3.01	-36.46	0.13	68	2
LallaRookh_6	39	5.1	-0.68	0.21	0.25	2.30	-36.35	0.12	76	3
LallaRookh_93013	44	0.8	-0.69	0.53	0.25	0.22	-35.68	0.07	66	1
LallaRookh_93014	43	1.0	-0.72	0.01	0.18	0.29	-36.48	0.09	50	1
LallaRookh_93015	29	3.2	-1.10	0.63	0.14	0.17	-34.87	0.01	54	3
LallaRookh_93016	27	1.4	-1.17	0.18	0.07	0.29	-35.31	0.00	32	2
LallaRookh_93017	42	3.8	-1.02	0.61	0.32	0.52	-37.17	0.09	89	3
LallaRookh_93018	50	4.4	-0.10	0.75	0.41	0.55	-32.73	0.06	96	4
LallaRookh_93019	40	2.4	-0.43	0.03	0.17	0.55	-35.61	0.08	49	2
LallaRookh_93020	33	0.1	-0.95	0.47	0.19	0.19	-36.13	0.10	65	0

Table 2: Lalla Rookh carbonate C and O isotopic data.

Sample	carb [%]	$\delta^{13}C$ [‰]	SD [‰]	$\delta^{18}O$ [‰]	SD [‰]
LallaRookh-1	3.6	-5.1		-17.8	
LallaRookh_2	20.3	-8.8	0.01	-20.4	0.17
LallaRookh-3	4.9	-6.8		-20.4	
LallaRookh_5	13.7	-8.8	0.01	-20.3	0.06
LallaRookh_6	4.5	-3.8	0.08	-19.8	0.11
LallaRookh_93020	4.8	-9.2	0.02	-20.4	0.11

Table 3: Literature sources of Mesoarchean organic carbon isotope data.

Unit	Age [Ga]	Data references	Comment
Lacustrine and brackish:			
Moodies Group	3.22	(Gamper et al., 2012)	Microbial mats within fluvio-marine sandstone (Heubeck and Lowe, 1994)
Farrell Quartzite	3.0	(House et al., 2013)	Contains pebble conglomerates and silicified evaporites (Grey and Sugitani, 2009), suggesting intermittent subaerial exposure and fluvial input. REE data are consistent with a brackish water composition (Sugahara et al., 2010).
Witwatersrand Spgp	2.87-2.94	(Guy et al., 2012; Stüeken et al., 2015)	Fluvial and deltaic facies from several formations (Guy et al., 2012)
Marine:			
Kromberg Fm	3.3	(Hayes et al., 1983; Strauss and Moore, 1992)	Shallow to deep marine sedimentation, volcanically influenced (Ransom et al., 1999)
Sheba Fm	3.25	(Hayes et al., 1983; Yamaguchi, 2002)	Turbidites on continental slope (Eriksson, 1980); turbiditic deep water (Zeh et al., 2013).
Cleaverville Fm	3.2	(Strauss and Moore, 1992)	Deep marine hydrothermally influenced sediments.
Dixon Island Fm	3.2	(Kiyokawa et al., 2006)	Hydrothermally influenced deep-water setting
Soanesville Gp	3.19	(Strauss and Moore, 1992; Stüeken et al., 2015)	Continental slope deposit
Pongola Spgp	2.9-3.1	(Hayes et al., 1983; Ono et al., 2006; Strauss and Moore, 1992)	Considered shallow and possibly restricted marine, but probably had continuous seawater input. More detailed analyses are needed to identify facies with freshwater input within this succession.
Witwatersrand Spgp	2.85-2.96	(Guy et al., 2012; Stüeken et al., 2015; Watanabe et al., 1997; Yamaguchi, 2002)	Marine facies distal to deltaic influence
Nullagine Gp	2.9	(Stüeken et al., 2015)	Deep-water depositional environment dominated by turbidites (Bagas et al., 2008; Nijman et al., 2010)
Nova Lima Gp	2.8	(Strauss and Moore, 1992)	Marine turbiditic sediments interbedded with BIF (Lobato et al., 1998; Machado et al., 1996)

Figure captions

Figure 1: Location of the Lalla Rookh sandstone in the Pilbara craton. Adapted from Krapež & Barley (1987). Star = location of the drill core (SRD-8).

Figure 2: Facies in the Lalla Rookh basin. (a) Map view of basin infill, adapted from Krapež & Barley (1987). Red star = drill core location. (b) Schematic cross-section of the Lalla Rookh Sandstone, showing facies distribution and continuity through sequence stacking, with red star showing drillcore location. Modified from Buick et al. (1998). Sequences 1-10 represent episodes of basin infill following rejuvenation events by vertical motion along the bounding faults.

Figure 3: Photomicrographs of the Lalla Rookh Sandstone. (a) Kerogenous lamina in silty shale, transmitted light; (b) Subrounded detrital pyrite (bright grains) within siltstone, reflected light; (c) Lag deposit of heavy detrital minerals (opaque grains) highlighted with dashed white line, transmitted light; (d) Same as (c) under reflected light, detrital pyrite stands out as bright grains.

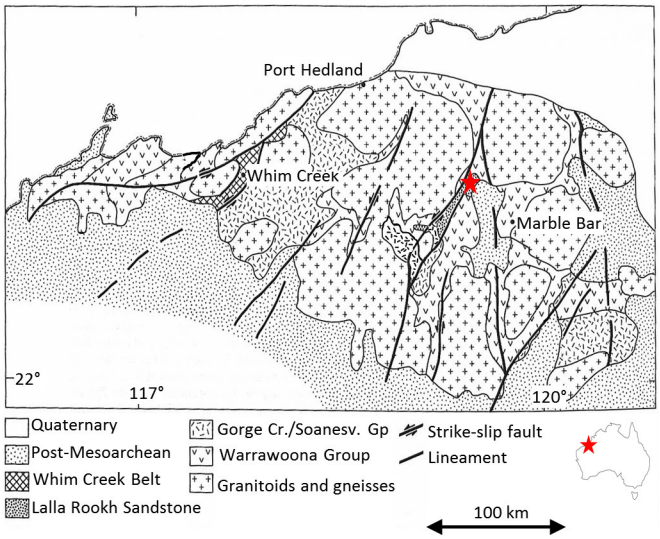
Figure 4: Mesoarchean CNO isotopes. (a) Organic C and total N. Lalla Rookh data comprise the non-marine, sub-greenschist category. Other data are from the literature (Stüeken et al., 2015; Yamaguchi, 2002). Dark blue shading = canonical N_2-fixation by FeMo-nitrogenase (nif); light blue shading = FeMo-based N_2 fixation in some thermophiles/with high Fe supply (Nishizawa et al., 2014; Zerkle et al., 2008). Horizontal dashed lines = minimum limits of CO_2 fixation by the Calvin cycle (-35‰) and by autotrophs using the acetyl-CoA pathway (-43‰, Zerkle et al., 2005). (b) Carbonate C and O isotopes in cements of the Lalla Rookh Sandstone. Gr = greenschist; sub-gr. = sub-greenschist.

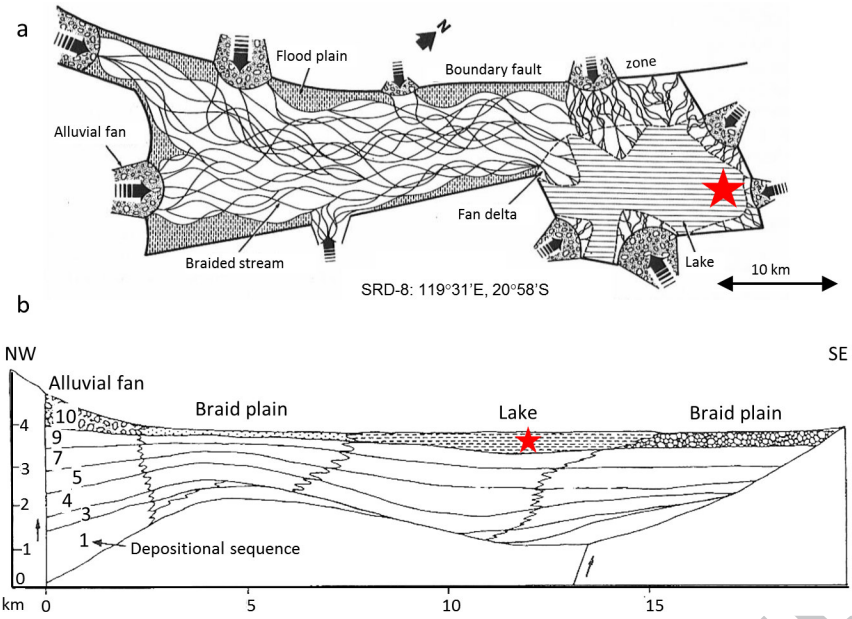
Figure 5: Scatter plots for organic carbon and bulk nitrogen isotopic data. a: Total nitrogen (TN) versus total organic carbon (TOC), showing the strong correlation between the two parameters. The positive TN-intercept likely reflects storage of N in silicate minerals during diagenesis (Schroeder and McLain, 1998). **b:** Bulk nitrogen isotopes ($\delta^{15}N_{bulk}$) versus ratios of organic carbon to total nitrogen (C/N). The absence of a correlation argues against strong metamorphic alteration of the nitrogen isotope data (Haendel et al., 1986). **c:** Bulk nitrogen isotopes versus total nitrogen content. The absence of a correlation indicates that the nitrogen isotopies are not skewed by one endmember. **d:** Organic carbon isotopes ($\delta^{13}C_{org}$) versus bulk nitrogen isotopes. The absence of a correlation argues against metasomatic fluid alteration (Schimmelmann and Lis, 2010; Schimmelmann et al., 2009).

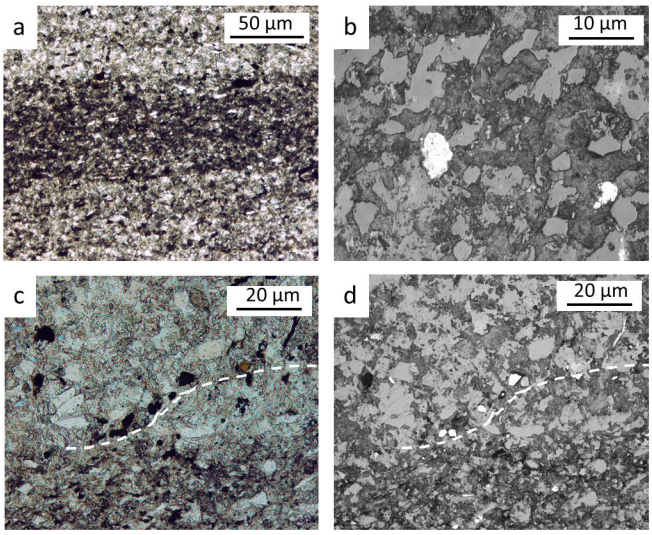
Figure 6: Carbonate content versus carbonate-carbon isotopes ($\delta^{13}C_{carb}$). The most carbonate-rich samples have among the most negative isotopic values, which is inconsistent with metamorphic equilibration between organic carbon and carbonate-carbon.

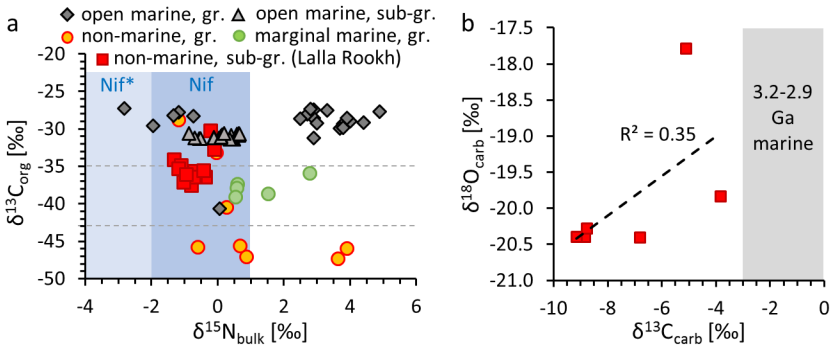
Figure 7: Reconstructed authigenic nitrogen isotopic ratios. The isotopic values were calculated by mass balance with the conservative assumption that the y-axis intercept in Fig. 5a (25ppm) is entirely detrital and has an isotopic composition of -1.3‰ (Fig. 5c). The blue shading indicates that the reconstructed authigenic $\delta^{15}N_{bulk}$ values fall mostly within the enzymatic N_2 fixation window.

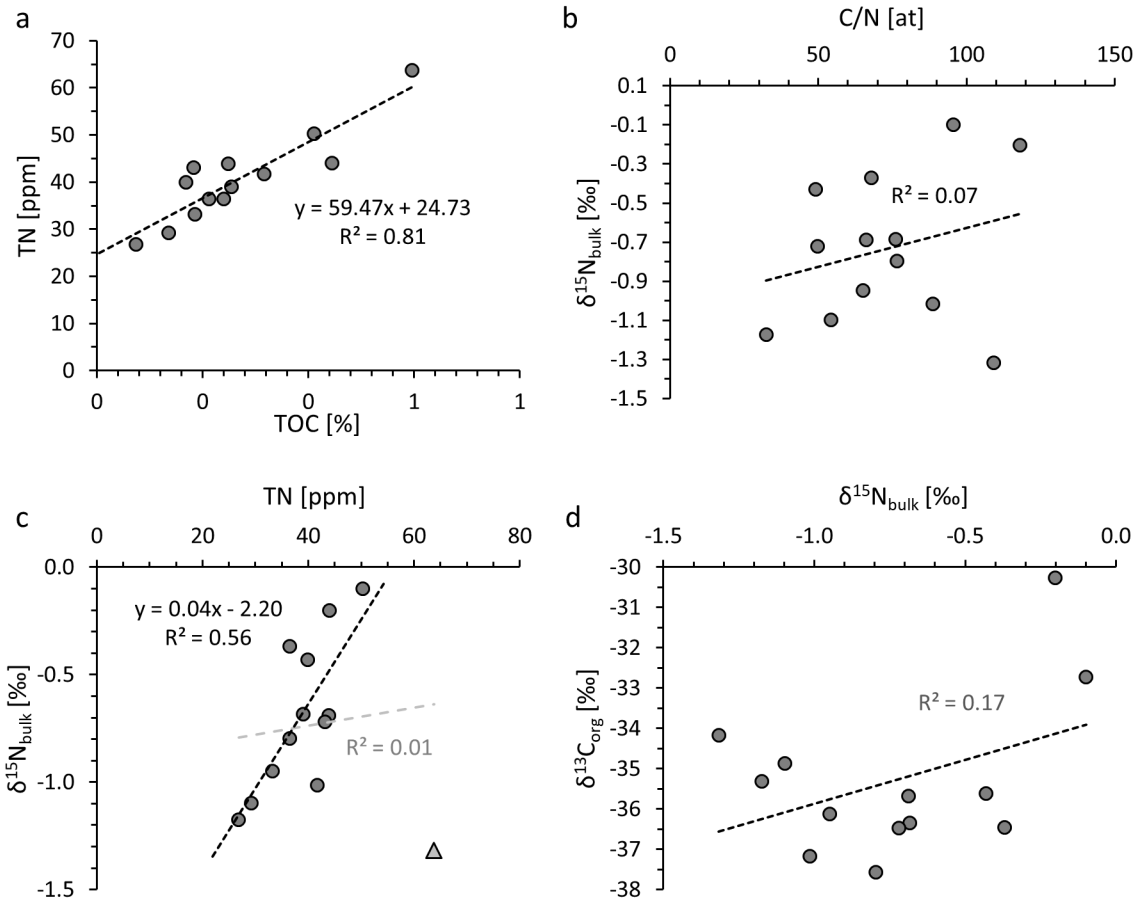
Figure 8: Organic carbon isotopes in the mid-Archean. Data from the Lalla Rookh Sandstone (this study) are included in the non-marine category at 3.0 Ga. See Table 3 for literature references. Dashed lines as in Fig. 4.

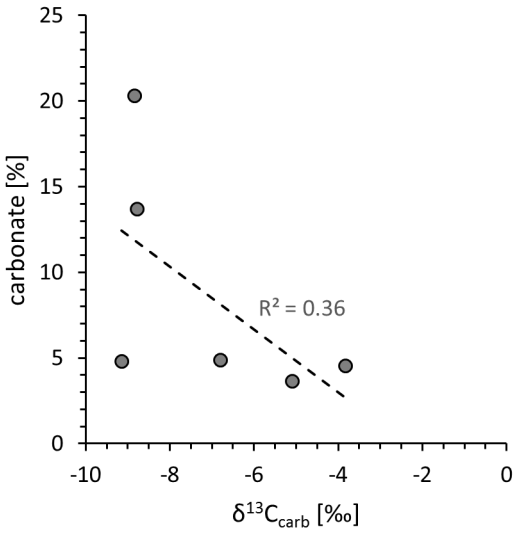


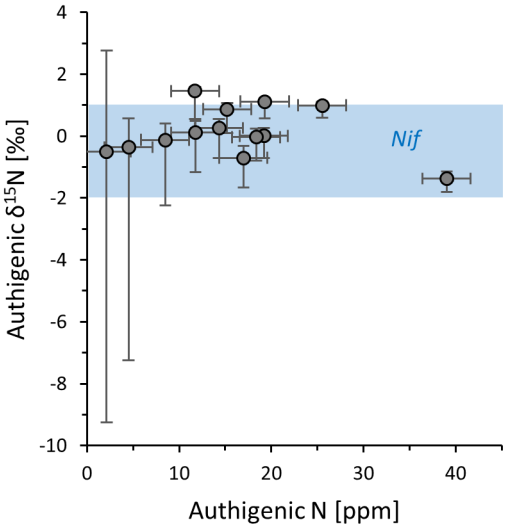


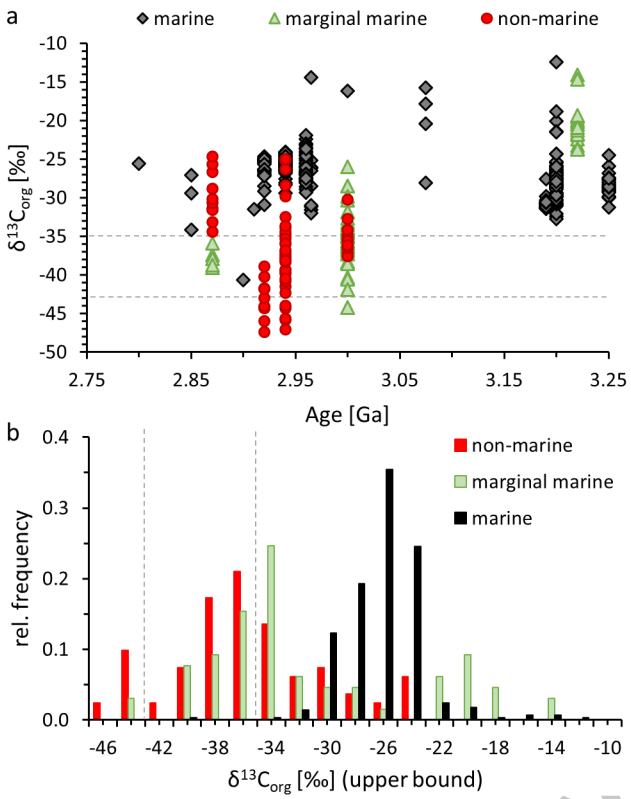












- The Lalla Rookh Sandstone records microbial life in lakes back to 3.0 Ga
- Nitrogen isotopes point to biological N_2 fixation in an anoxic setting
- Organic $\delta^{13}C$ values in this and other Mesoarchean lakes are lighter than in the ocean
- Lakes may have contributed to Archean hydrocarbon haze formation
- Early continents may have offered niches for the diversification of microbial life